UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 22, 2018

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Environmental Risk Assessment for a FIFRA Section 3 Registration of two products, Bacteriophages active against *Xylella fastidiosa* Technical Manufacturing Use Product and OTC-821 End Use Product, Containing the New Active Ingredient Bacteriophages active against *Xylella fastidiosa*; EPA File Symbols 92918-E and 92918-R; PC Code 116404; Decision Nos. 527226 and 527227; Submission Nos. 1011100 and 1000665; DP Barcodes: 444185 and 439425; MRID 50550101

FROM: Sarah Butler, Biologist
Risk Assessment Branch
Biopesticides and Pollution Prevention Division

THROUGH: Shannon Borges, Senior Scientist
Risk Assessment Branch
Biopesticides and Pollution Prevention Division

TO: Alexandra Boukedes, Risk Manager
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division

I. Executive Summary

Otsuka Pharmaceutical Company, Ltd has submitted an application for a Section 3 registration of a technical product (Bacteriophages active against *Xylella fastidiosa*; EPA File Symbol 92918-E) containing a new active ingredient, Bacteriophages active against *Xylella fastidiosa*. One end use product (EP), OTC-821, EPA File Symbol 92918-R, has been proposed containing this active ingredient which will contain 0.00028% Bacteriophages active against *Xylella fastidiosa*. The minimum viability of the bacteriophages in this EP is 1 X 10^{10} plaque -forming units (pfu) per mL. This EP is proposed for use to control Pierce's Disease in grapevines, which is caused by the bacterial pathogen *Xylella fastidiosa*, and the proposed application method for this EP is injection directly into grapevines at 2-4 injection sites per vine cordon. The application rate ranges from 40 to 120 microliters per injection, with 2-3 applications per season depending on disease pressure.

The bacteriophages that make up this active ingredient (AI) have been isolated from plant debris and soil, and are specific to *X. fastidiosa*. These bacteriophages will control *X. fastidiosa* infections in grape vines through a process known as the lytic cycle. During this process, bacteria

cells are infected by phage particles, which use the cell's machinery to produce nucleic acids and proteins needed for production of new phages, ultimately resulting in the lysis and death of the host bacteria cells.

Because the EP proposed for registration will only be applied via injection, and the phage particles are not expected to travel far from the site of injection (Das et al. 2015). Some exposure to terrestrial nontarget organisms may be possible as a result of their chewing of plant parts or consumption of nectar and/or pollen near the site of injection. However, this exposure, if it occurs, is expected to be very limited because of the limited application amount and movement within the plant. Nonetheless, if exposure does occur, it is not expected to result in adverse effects in nontarget organisms. Otsuka Pharmaceutical Company submitted rationale to satisfy all nontarget organism data requirements, which presented several arguments for why adverse effects to nontarget organisms are not expected to result from proposed labeled applications of Bacteriophages active against *Xylella fastidiosa*. One of the strongest of the arguments presented in this rationale is the fact that the bacteriophages proposed for registration are strictly lytic, meaning that all phage particles will enter the lytic cycle as described above.

The EPA has reviewed the submitted rationale and labels, and has conducted literature searches for relevant scientific literature on Bacteriophages active against *Xylella fastidiosa* and has determined that adverse effects to nontarget organisms resulting from proposed labeled applications of this AI are not expected. Since EPA has determined that no effects are anticipated for any non-target species exposed to Bacteriophages active against *Xylella fastidiosa* as a result of the proposed labeled applications, effects to federally listed threatened and endangered ('listed') species and their designated critical habitats are also not expected. Therefore, a "No Effect" determination is made for direct and indirect effects to listed species and their designated critical habitats resulting from the proposed uses of Bacteriophages active against *Xylella fastidiosa* as labeled.

II. Introduction

Otsuka Pharmaceutical Company, Ltd has submitted an application for a Section 3 registration of a technical product (Bacteriophages active against *Xylella fastidiosa*; EPA File Symbol 92918-E) containing a new active ingredient, Bacteriophages active against *Xylella fastidiosa*. This AI is composed of bacteriophages isolated from plant debris and soil and is proposed for use to control Pierce's Disease in grapevines, which is caused by the bacterial pathogen *Xylella fastidiosa*. The bacteriophages that make up this AI are specific to *X. fastidiosa*, and kill this bacterium by infecting bacteria cells and using the cell's machinery to produce nucleic acids and proteins needed for production of new phage particles. This process, known as the lytic cycle, eventually causes the bacterial host cell to rupture and die while the mature bacteriophages are released to infect other *S. fastidiosa* cells.

One end use product (EP), OTC-821, EPA File Symbol 92918-R, has been proposed containing this active ingredient. This product will contain 0.00028% bacteriophage, and the minimum viability of the bacteriophages in this EP is 1 X 10^{10} (pfu) per mL.

III. Nontarget Organism Exposure

The proposed application method for this EP is injection directly into grapevines at 2-4 injection sites per vine cordon depending on the size of the vine. The application rate ranges from 40 to 120 microliters per injection, with 2-3 applications per season depending on disease pressure. Because the only application method proposed for this EP is injection directly into grapevines, exposure to nontarget organisms is expected to be very minimal. Furthermore, research indicates that the phages will only distribute about 50 cm from the place of injection, and once the bacterial host population has been decimated by the phage, the phage population will decrease to around 10-100 PFU/gram of tissue in each cordon (Das et al. 2015). Thus, if parts of a plant that has been injected with bacteriophages are consumed by a nontarget organism, very few, if any, bacteriophages are likely to be consumed by that organism.

A. Terrestrial Environments

1. Birds, Mammals, Nontarget Insects and Honey Bees

Terrestrial animals may be exposed to this bacteriophage as a result of consumption of treated parts of grape vines. However, because the grapes themselves will not be directly treated, and the phage particles are not expected to travel far from the site of application, it is not likely that consumption of grapes will result in exposure to this AI. It is possible that there could be some exposure to mammals and nontarget insects through chewing of plant parts close to the site of injection, and exposure of insects and honey bees through consumption of nectar and/or pollen near the site of injection. However, given the limited application amount and limited movement within the plant, exposure is expected to be very limited if it occurs at all. However, it is unlikely to be above natural levels of bacteriophage exposure, as bacteriophages are the most abundant organisms in the biosphere, outnumbering bacteria 10 – 100-fold (Gill and Young 2011). In addition, it is estimated that soil contains 100 million or more bacteriophages per gram (Gil and Abedon 2003).

2. Nontarget Plants

Because the application method proposed for this EP is injection directly into grapevines, exposure to Bacteriophages active against *Xylella fastidiosa* is very unlikely in nontarget plants as a result of proposed applications of this AI.

B. Aquatic Environments

Exposure to Bacteriophages active against *Xylella fastidiosa* in the aquatic environment is not expected to occur because, as stated earlier, the proposed EP containing this active ingredient will only be applied to grapevines via injection. However, in the unlikely chance that exposure does occur, such exposure would not be above naturally occurring levels of bacteriophages in aquatic environments. As stated previously, bacteriophages are the most abundant organisms in the biosphere, outnumbering bacteria 10 – 100-fold (Gill and Young 2011). In addition, the density of viruses in estuarine waters is approximately 10^7 particles/mL with the majority of these virus particles being bacteriophages (Wommack and Colwell 2000).

IV. Summary of Nontarget Effects Data

Table 1 provides the status of the data requirements as published in 40 CFR § 158.2150 for Bacteriophages active against *Xylella fastidiosa* for ecological risk assessment. Scientific rationale and public literature studies were submitted to satisfy all nontarget organism testing. Information from the scientific rationale and literature submission is included in the section below, and a Data Evaluation Record is attached.

The information provided is sufficient to satisfy the Tier I nontarget organism data requirements for ecological risk assessment for the active ingredient. Further testing of nontarget organisms at higher tiers is not required for the current label uses.

Table 1. Summary of data submitted to comply with nontarget organism data requirements published in 40 CFR § 158.2150 for support of the registration of Bacteriophages active against *Xylella fastidiosa*.

Data Requirement	OCSPP Guideline No.	Results Summary and Classification	MRID No.
Avian Oral Toxicity, Avian Inhalation Toxicity, Wild Mammal Testing, Freshwater Fish Testing, Freshwater Invertebrate Testing, Marine/Estuarine Animal Testing, Nontarget Plant Testing, Nontarget Insect Testing, Honey Bee Testing	885.4050, 885.4100, 885.4150, 885.4200, 885.4240, 885.4280, 885.4300, 885.4340, 885.4380	Data waiver rationale provides sufficient information to determine that toxicity/pathogenicity to nontarget organisms is not expected. **Classification: Acceptable**	50550101

Scientific rationale was sufficient to conclude that adverse effects are not expected in nontarget organisms as a result of exposure to Bacteriophages active against *Xylella fastidiosa*.

V. Literature Search Results

BPPD conducted literature searches to assess potential effects to nontarget organisms that could result from proposed applications of the EP containing Bacteriophages active against *Xylella fastidiosa*. These searches were conducted in February of 2018, and used the Web of Science database, which includes articles published from 1970 to the present. Searches were conducted using the Web of Science Core Collection, the default database within the Web of Science system. Searches were conducted using the terms "Bacteriophage *Xylella fastidiosa* and avian," "Bacteriophage *Xylella fastidiosa* and insects," "Bacteriophage *Xylella fastidiosa* and mammals," "Bacteriophage *Xylella fastidiosa* and terrestrial plants," and "Bacteriophage *Xylella fastidiosa* and aquatic organisms." No results were returned for any of these searches.

VI. Ecological Risk Characterization

The scientific rationale submitted by Otsuka Pharmaceutical Company, Ltd (MRID 50550101) explains that exposure will be significantly limited by the application method, as the only application method for the EP containing this active ingredient is injection directly into grapevines. In addition, the scientific rationale cites a study by Das et al. (2015), which demonstrates that the phages currently proposed for registration will only distribute about 50 cm from the place of injection, and once the bacterial host population has been decimated by the phage, the phage population will decrease to around 10-100 PFU/gram of tissue.

A study by Ahern et al. (2014) is cited, which described the isolation and gene sequencing of the first virulent phages for *Xylella fastidiosa.* This study confirmed that like most bacteriophages, the bacteriophages present in this AI are specific to *X. fastidiosa*, and thus will not cause adverse effects to non-target organisms. This study also determined that genes associated with lysogeny were not present in these phages, meaning that they lack the genetic factors required for integration into the bacterial genome.

The literature cited in the rationale also shows that bacteriophages are ubiquitous in the natural environment. As stated previously, bacteriophages are the most abundant organisms in the biosphere (Gill and Young 2011). They are prevalent in lake and marine waters, as well as soil, buds, leaves, root nodules, roots, rotting fruit, seeds, and stems of plants. As a result, birds, mammals, insects, terrestrial plants, and aquatic species are exposed to phages throughout their natural environment.

The rationale for avian oral Toxicity/Pathogenicity and avian inhalation Toxicity/Pathogenicity also cited several studies from the scientific literature which assessed the efficacy of using lytic bacteriophages to control harmful bacteria including *Escherichia coli* O78:K80 and species of *Campylobacter*, in poultry. In these studies, chickens were treated with bacteriophages of *Escherichia coli* O78:K80 or bacteriophages of a pathogenic *Campylobacter* species and were also challenged with the related pathogenic bacterial strain. While bacteriophage treatments did not completely eliminate bacterial infections, these treatments did reduce the severity of the infections, suggesting that bacteriophage treatments are at least somewhat effective in treating bacterial infections in poultry. In addition, no adverse effects of the bacteriophages were reported in these studies, which Otsuka Pharmaceutical Company argues supports their claim that Bacteriophage active against *X. fastidiosa* will not be toxic to birds.

The rationale for Toxicity/Pathogenicity to wild mammals cited two additional studies which assessed the effects of treating rats orally with lytic bacteriophages (Chibani-Chennoufi et al. 2004; Carlton et al. 2005). No adverse health effects were observed in rats treated with bacteriophages compared to controls in either of these studies, indicating that consumption of phages does not pose any risk to mammals. The rationale for Freshwater Fish testing and Marine/Estuarine Fish and Invertebrate Testing demonstrated that phages are also well tolerated by aquatic organisms under experimental conditions. To support this rationale, a review article by Richards (2014) was cited which discusses several studies on the use of bacteriophages in aquaculture as a substitute for antibiotics. Some of the studies examined phage therapies for diseases and associated pathogens of freshwater fish and shellfish, while other studies examined

phage therapies for diseases and associated pathogens of marine animals. No adverse effects were observed in these studies, which suggests that bacteriophages are generally not toxic to aquatic animals.

BPPD has determined that this rationale is acceptable for assessment of risk to nontarget organisms from exposure to Bacteriophage active against *X. fastidiosa*. Based on this rationale, adverse effects to nontarget organisms are not expected to result from proposed label applications of these Bacteriophages. The proposed EP containing this AI will be applied via injection directly into grapevines, thus significantly limiting exposure, and the natural prevalence and the ecology of bacteriophages make adverse effects in nontarget organisms unlikely in the case that exposure to this AI does occur.

The lytic nature of the bacteriophages proposed for registration is particularly important in terms of minimizing risk to nontarget organisms. Lytic bacteriophages always begin a process known as the lytic cycle after they bind to a host cell, during which they take over the machinery of the host cell to produce the nucleic acids and proteins needed for production of new phage particles, a process which results in the lysis and death of the bacterial host cell. This is in contrast to temperate phages, which go through the lysogenic cycle and do not immediately lyse the host bacteria cell. Instead, temperate phages integrate their genome into that of the host cell, which can exacerbate pathogenicity and/or virulence of the host bacteria, potentially causing the bacteria to become pathogenic to their host organism. Although the public literature studies on intentional exposure of poultry, rats, and aquatic organisms to bacteriophages did not use the particular bacteriophages contained in this AI, these studies do provide further evidence that exposure to bacteriophages in general is not likely to result in adverse effects in nontarget organisms. Because the bacteriophages in this AI are extremely host-specific, it is not reasonable to expect to find studies in which nontarget organisms were exposed to these specific phages.

VII. Risk to Federally Listed Threatened and Endangered Species

Since EPA has determined that no effects are anticipated for any non-target species exposed to Bacteriophages active against *Xylella fastidiosa*, as a result of the proposed labeled applications, effects to federally listed threatened and endangered ('listed') species and their designated critical habitats are also not expected. Therefore, a "No Effect" determination is made for direct and indirect effects to listed species and their designated critical habitats resulting from the proposed uses of Bacteriophages active against *Xylella fastidiosa*, as labeled.

VIII. References

Ahern, S.J., Das, M., Bhowmick, T.S., Young, R., and Gonzalez, C.F. (2014). Characterization of novel virulent broad-host-range phages of *Xylella fastidiosa* and *Xanthomonas*. Journal of bacteriology 196(2):459-471.

Carlton, R.M., Noordman, W.H., Biswas, B., De Meester, E.D., and Loessner, M.J. (2005). Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence,

bioinformatic analyses, oral toxicity study, and application. Regulatory Toxicology and Pharmacology 43(3): 301-312.

Chibani-Chennoufi, S., Sidoti, J., Bruttin, A., Kutter, E., Sarker, S., and Brüssow, H. (2004). In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. Antimicrobial agents and chemotherapy 48(7): 2558-2569.

Das, M., Bhowmick, T.S., Ahern, S.J., Young, R., and Gonzalez, C.F. (2015). Control of Pierce's disease by phage. PloS one 10(6): e0128902.

Gill, J., and Young, R. (2011). Therapeutic Applications of Phage Biology: History, Practice, and Recommendations. Chapter 17 in A.A. Miller and P.F. Miller (eds.), *Emerging Trends in Antibacterial Discovery: Answering the Call to Arms*. Caister Academic Press, Norfolk, UK.

Richards, G.P. (2014). Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology. Bacteriophage 4(4): e975540.

Wommack, K.E., & Colwell, R.R. (2000). Virioplankton: viruses in aquatic ecosystems. Microbiology and molecular biology reviews 64(1): 69-114.

Primary Reviewer: Sarah Butler, EPA/BPPD/RAB SLB **Date:** 3/22/18

Secondary Reviewer: Shannon Borges, EPA/BPPD/RAB **Date:** 3/22/18

DATA EVALUATION RECORD

REQUIREMENT: U.S. EPA OCSPP Guideline: 885.4050–Avian Oral Toxicity
U.S. EPA OCSPP Guideline: 885.4100–Avian Inhalation Toxicity
U.S. EPA OCSPP Guideline: 885.4150–Wild Mammal Testing
U.S. EPA OCSPP Guideline: 885.4200–Freshwater Fish Testing
U.S. EPA OCSPP Guideline: 885.4240–Freshwater Invertebrate Testing
U.S. EPA OCSPP Guideline: 885.4280–Marine/Estuarine Animal Testing
U.S. EPA OCSPP Guideline: 885.4300–Nontarget Plant Testing
U.S. EPA OCSPP Guideline: 885.4340–Nontarget Insect Testing
U.S. EPA OCSPP Guideline: 885.4380–Honey Bee Testing

TEST MATERIAL: Bacteriophage active against *Xylella fastidiosa*

CITATION: Leslie E. Patton, (Technology Sciences Group). 2018 Revised Response to Tier 1 Microbial Pesticide Data Requirements for Bacteriophage active against *Xylella fastidiosa*. Sponsored by Otsuka Pharmaceuticals Co., Ltd., 26 Davis Dr. PO Box 13528 Research Triangle Park, NC 27709. November 8, 2016. Unpublished MRID No. 50550101 (replaces MRID 50159303).

SPONSOR: Otsuka Pharmaceuticals Co., Ltd., 2-9, Kanda-Tsukasamachi, Chiyoda-ku, Tokyo 101-8535, Japan

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. The study was not conducted in compliance with GLP [40 CFR § 160]. The study is not required to be GLP, since it is a waiver request. This DER does not contain FIFRA CBI.

CLASSIFICATION: ACCEPTABLE

I. OCSPP 885.4050, 885.4100, 885.4150, 885.4200, 885.4240, 885.4280, 885.4300, 885.4340, 885.4380 – Avian Oral Toxicity, Avian Inhalation Toxicity, Wild Mammal Testing, Freshwater Fish Testing, Freshwater Invertebrate Testing, Marine/Estuarine Animal Testing, Nontarget Plant Testing, Nontarget Insect Testing, Honey Bee Testing

A. **RATIONALE:** Otsuka Pharmaceuticals Co, Inc. requested consideration of existing data in satisfying the requirement for all nontarget organism toxicity/pathogenicity testing. The following information was submitted to fulfill all data requirements: 1) the proposed use pattern will not result in significant exposure of nontarget organisms to the TGAI, 2) life history traits of this phage make adverse health effects in nontarget organisms very unlikely, 3) nontarget organisms are regularly exposed to bacteriophages in their environment, and 4) phages do not persist in the environment.

Otsuka supports this rationale by explaining that the application method for the proposed EP is injection directly into grape plants, and therefore, residues will not be left on plants or soil where they could be

ingested by nontarget organisms. In addition, results from a study by Das et al. (2015) suggests that the phages will only distribute about 50 cm from the place of injection, and once the bacterial host population has been decimated by the phage, the phage population will decrease to around 10-100 PFU (plaque forming units) per gram of tissue in each cordon. As a result, Otsuka argues that if a nontarget organism consumed part of a treated plant, the exposure to the phage would be very low.

Otsuka also discusses three lifestyle traits of the phages proposed for registration which make adverse health effects in nontarget organisms very unlikely: the host-specific nature of the phage, the phage has been isolated and cultured to be strictly lytic, and phage concentrations are self-regulating. To illustrate these characteristics, Otsuka cites a study by Ahern et al. (2014) which described the isolation and characterization of the first virulent phages for *Xylella fastidiosa*. Ahern et al. (2014) also sequenced the genes of these phages and determined that genes associated with lysogeny were not present. To illustrate the third characteristic, Otsuka cites a study by Abedon and Thomas-Abedon (2010) as well as the previously mentioned study by Das et al. (2015).

Regarding exposure, several studies are cited which demonstrate the natural occurrence of phages in lake and marine waters as well as soil, buds, leaves, root nodules, roots, rotting fruit, seeds, and stems of plants (Eayre et al., 1995; Chibani-Chennoufi et al. 2004b; Wommack and Colwell 2000). In addition, Gil and Abedon (2003) estimated that soil contains 100 million or more bacteriophages per gram. Otsuka also states that phages are the most abundant organisms in the biosphere, and that they outnumber bacteria 10 – 100-fold (Gill and Young 2011). Finally, regarding the persistence of phages in the environment, Otsuka cites two studies that illustrate that UV radiation from sunlight significantly reduces bacteriophage populations on plant surfaces (Iriarte et al. 2007; Jones et al. 2012). Desiccation and temperature were also found to reduce bacteriophage populations on tomato leaves but to a lesser extent than UV radiation (Iriarte et al. 2007).

B. **EPA REVIEW:** The reviewer agrees with the rationale presented. The injection method of application is expected to significantly reduce the potential for nontarget organisms to be exposed to the bacteriophages, and the three general characteristics of bacteriophages make it very unlikely that nontarget organisms would be affected if they were to come into contact with these bacteriophages. The strictly lytic nature of the bacteriophages proposed for registration is particularly advantageous in terms of minimizing risk to nontarget organisms. This is because, lytic bacteriophages always begin a process known as the lytic cycle after they bind to a host cell. During this cycle, the bacteriophages take over the machinery of the host cell to produce the nucleic acids and proteins needed for production of new phage particles, a process which results in the lysis and death of the bacterial host cell. This is in contrast to temperate phages, which integrate their genome into that of the host cell and do not necessarily kill them, but can exacerbate pathogenicity and/or virulence of the host bacteria, potentially causing the bacteria to become pathogenic to their host organism.

II. OCSPP 885.4050, 885.4100 – Avian Oral Testing, Avian Inhalation Testing

A. **RATIONALE:** In addition to the information provided in section I above, Otsuka Pharmaceuticals Co, Inc. submitted the following information to satisfy the requirement for an Avian Oral Toxicity/Pathogenicity Test (OCSPP 885.4050) and an Avian Inhalation Toxicity/Pathogenicity test (OCSPP 885.4100): phages are well tolerated by birds under experimental conditions.

Otsuka supported this rationale by citing several studies which assessed the efficacy of using lytic bacteriophages to control harmful bacteria in poultry, including *Escherichia coli* O78:K80 (Lau et al. 2010; Huff et al. 2005; Huff et al. 2002) and species of *Campylobacter* (Kittler et al. 2013; El-Shibiny et

al. 2005). Otsuka argued that the absence of reported adverse effects in these studies supports their claim that Bacteriophage active against *X. fastidiosa* will not be toxic to birds.

B. **EPA REVIEW:** The reviewer agrees with the rationale presented. However, it should be noted that while the papers by Lau et al. (2010), Huff et al. (2005), Huff et al. (2002), and Kittler et al. (2013) described experiments in which chickens were intentionally exposed to *Escherichia coli* or *Campylobacter* and related bacteriophages, the paper by El-Shibiny et al. (2005) described a study in which naturally occurring *Campylobacters* and *Campylobacter*-specific bacteriophages were isolated and enumerated during the rearing cycle of free-range and organic chickens. Nevertheless, this rationale does provide valuable information to help satisfy the avian data requirements.

III. OCSPP 885.4150 – Wild Mammal Testing

A. **RATIONALE:** In addition to the information provided in section I above, Otsuka Pharmaceuticals Co, Inc. submitted the following information to fulfill the data requirement for Wild Mammal Testing (OCSPP 885.4150): phages are well tolerated by mammals under experimental conditions.

Otsuka supported this rationale by citing two studies which assessed the effects of phages on rats. In one study, adult mice were exposed orally to four phages which had been added to their drinking water, and no histopathological changes of the gut mucosa were detected (Chibani-Chennoufi et al. 2004a). The other study was a repeated dose oral toxicity study, in which rats were exposed to five doses (one dose of 1.0 ml per day for five days) of phage P100, a phage which can infect and kill a majority of *Listeria monocytogenes* strains (Carlton et al. 2005). No abnormal histological changes, morbidity or mortalities were observed, indicating that consumption of this phage does not pose any risks to mammals.

B. **EPA REVIEW:** The reviewer agrees with the rationale presented. Based on this rationale, adverse effects are not expected to occur in wild mammals as a result of exposure to Bacteriophage active against *Xylella fastidiosa.*

IV. OCSPP 885.4200, 885.4280 – Freshwater Fish Testing, Marine/Estuarine Fish and Invertebrate Testing

A. **RATIONALE:** In addition to the information provided in section I above, Otsuka Pharmaceuticals Co, Inc. submitted the following information to fulfill the data requirements for Freshwater Fish Toxicity/Pathogenicity (OCSPP 885.4200) and Marine/Estuarine Fish and Invertebrate Testing (OCSPP 885.4280): phages are well tolerated by aquatic organisms under experimental conditions.

To support this rationale, Otsuka cites a review article by Richards (2014) which discusses several studies on the use of bacteriophages in aquaculture as a substitute for antibiotics. Some of the studies examined phage therapies for diseases and associated pathogens of freshwater fish and shellfish including: hemorrhagic septicemia (*Aeromonas hydrophila*) in loaches, furunculosis (*Aeromonas salmonicida*) in trout and salmon, columnaris disease (*Flavobacterium columnare*) in catfish, rainhow trout fry syndrome or cold water disease (*Flavobacterium psychrophilum*) in trout and salmon, ulcerative skin lesions (*Pseudomonas aeruginosa*) in freshwater catfish, bacterial hemorrhagic ascites disease (*Pseudomonas plecoglossicida*) in ayu fish. Other studies examined phage therapies for diseases and associated pathogens of marine animals, including: edwardsiellosis (*Edwardsiella tarda*) in eel, lactococcosis (*Lactococcus* spp.) in yellowtail, streptococcosis (*Streptococcus iniae*) in flounder, and luminescent vibriosis (*Vibrio harveyi*) in shrimp. No adverse effects were observed in these studies, which suggests that bacteriophages are generally not toxic to aquatic organisms.

B. EPA REVIEW: The reviewer agrees with the rationale presented.

V. OCSPP 885.4300 – Nontarget Plant Testing

A. RATIONALE: In addition to the information provided in section I above, Otsuka Pharmaceuticals Co, Inc. submitted the following information to fulfill the data requirements for Nontarget Plant Testing (OCSPP 885.4300): grapevines were not adversely affected by inoculation with the proposed TGAI.

To support this rationale, Otsuka cites a study designed to test the efficacy of the TGAI against Pierce's Disease in grapevines (*Vitus vinifera*). In this study, grapevines were inoculated with phage cocktail at a concentration of 10^{10} PFU/ml either alone, after inoculation with *Xylella fastidiosa* subsp. *fastidiosa* strain Temecula 1 (Xf-T1), or before the Xf-T1 inoculation (Das et al. 2015). The grapevines were evaluated regularly for 12 weeks for phage and bacteria concentration as well as signs of Pierce's Disease. The researchers determined that the TGAI was effective against Pierce's Disease and reduced Xf-T1 levels. No qualitative signs of poor health were observed, indicating that the phage was not toxic to the plants

B. EPA REVIEW: The reviewer agrees with the rationale presented.

References

Abedon, S.T., & Thomas-Abedon, C. (2010). Phage therapy pharmacology. Current pharmaceutical biotechnology 11(1): 28-47.

Ahern, S.J., Das, M., Bhowmick, T.S., Young, R., and Gonzalez, C.F. (2014). Characterization of novel virulent broad-host-range phages of *Xylella fastidiosa* and *Xanthomonas*. Journal of bacteriology 196(2):459-471.

Carlton, R.M., Noordman, W.H., Biswas, B., De Meester, E.D., and Loessner, M.J. (2005). Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regulatory Toxicology and Pharmacology 43(3): 301-312.

Chibani-Chennoufi, S., Sidoti, J., Bruttin, A., Kutter, E., Sarker, S., and Brüssow, H. (2004a). In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. Antimicrobial agents and chemotherapy 48(7): 2558-2569.

Chibani-Chennoufi S., Bruttin, A., Dillmann, M.L., and Brussow H. (2004b). Phage-host interaction: an ecological perspective. Journal of bacteriology 186(12): 3677-3686.

Das, M., Bhowmick, T.S., Ahern, S.J., Young, R., and Gonzalez, C.F. (2015). Control of Pierce's disease by phage. PloS one 10(6): e0128902.

Eayre, C.G., Bartz, J.A. and Concelmo, D.E. (1995). Bacteriophages of *Erwinia carotovora* and *Erwinia ananas* isolated from freshwater lakes. Plant disease 79(8): 801-804.

El-Shibiny, A., Connerton, P.L., and Connerton, I.F. (2005). Enumeration and diversity of *campylobacters* and bacteriophages isolated during the rearing cycles of free-range and organic chickens. Applied and environmental microbiology 71(3): 1259-1266.

Gill, J., & Abedon, S.T. (2003). Bacteriophage ecology and plants. APSnet Feature.

Gill, J., and Young, R. (2011). Therapeutic Applications of Phage Biology: History, Practice, and Recommendations. Chapter 17 in A.A. Miller and P.F. Miller (eds.), *Emerging Trends in Antibacterial Discovery: Answering the Call to Arms*. Caister Academic Press, Norfolk,UK.

Huff, W.E., Huff, G.R., Rath, N.C., Balog, J.M., and Donoghue, A.M. (2002). Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. Poultry Science 81(10): 1486-1491.

Huff, W.E., Huff, G.R., Rath, N.C., Balog, J.M., and Donoghue, A.M. (2005). Alternatives to antibiotics: utilization of bacteriophage to treat colibacillosis and prevent foodborne pathogens. Poultry science 84(4): 655-659.

Iriarte, FB, Balogh, B, Momol, MT, Smith, LM, Wilson, M, Jones, JB. (2007). Factors affecting survival of bacteriophage on tomato leaf surfaces. Applied AND environmental microbioligy 73(6): 1704-1711.

Jones, JB, Vallad, GE, Iriarte, FB, et al. (2012). Considerations for using bacteriophages for plant disease control. Bacteriophage 2(4): e23857.

Kittler, S., Fischer, S., Abdulmawjood, A., Glünder, G., and Klein, G. (2013). Effect of bacteriophage application on *Campylobacter jejuni* loads in commercial broiler flocks. Applied and environmental microbiology 79(23): 7525-7533.

Lau, G.L., Sieo, C.C., Tan, W.S., Hair-Bejo, M., Jalila, A., and Ho, Y.W. (2010). Efficacy of a bacteriophage isolated from chickens as a therapeutic agent for colibacillosis in broiler chickens. Poultry science 89(12): 2589-2596.

Richards, G.P. (2014). Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology. Bacteriophage 4(4): e975540.

Wommack, K.E., & Colwell, R.R. (2000). Virioplankton: viruses in aquatic ecosystems. Microbiology and molecular biology reviews 64(1): 69-114.